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Evidence for Selection Against Heterozygotes: Post-Settlement Excess of Allozyme Homozygosity in a Cohort of the Chilean Oyster, *Ostrea chilensis* Philippi, 1845

J. E. TORO AND A. M. VERGARA

Instituto de Biología Marina, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

Reports of heterozygote deficiencies in electrophoretic surveys carried out in marine bivalves abound in the literature (1–6), but the mechanism or mechanisms producing this phenomenon have not been well defined. We report that, in the Chilean oyster (Ostrea chilensis)*, heterozygote deficiencies in a cohort obtained by mass spawning in the laboratory are not randomly distributed in time among genotypes. The eggs of the Chilean oyster are internally fertilized, and the larvae, which are brooded within the mantle cavity, have limited dispersal capabilities because of their extremely short pelagic stage (7). These features could allow mechanisms such as inbreeding or Wahlund effect to produce heterozygote deficiencies. However, we observed no significant heterozygote deficiencies in juveniles at 6 months of age; instead allozyme heterozygosity decreased over time. Inbreeding, Wahlund effect, aneuploidy, and null alleles are unlikely to be main causes of the heterozygosity deficiency in this cohort; if they were, the deficiency should be evident from the juvenile stage and would not necessarily increase over time (2, 5, 8, 9, 10). We suggest that selection against heterozygotes is the most probable cause of the increasing degree of heterozygote deficiency with age in this cohort of* O. chilensis*, a proposition that accords with data for other marine bivalve species (2, 4, 11).*

Populations of marine bivalves exhibit deficiency of allozyme heterozygotes. This deficiency has been demonstrated in laboratory studies of mussels and clams (2, 3), in studies using wild populations (8, 12) of *Mytilus edulis*, and in several studies of oysters *(Crassostrea virginica)* (13–15). In the Chilean oyster *(Ostrea chilensis)* a heterozygote deficiency was found in the carbonic anhydrase (CA) locus from a southern population (Melinka, 43°53′S) (1). The time at which heterozygote deficiency first appears in the population can help distinguish causative mechanisms (16). In laboratory studies with mussels, an overall significant deficiency of heterozygotes was found at the juvenile stage but not at the spat stage (4).

In the present study, we used a cohort of *O. chilensis* settled on artificial collectors in the Quempillén hatchery, Ancud, Chiloé (45°52′S, 73°46′W). The parental stock was a cohort of *O. chilensis* collected during December 1987 from a natural spatfall in the wild population at Quempillén estuary. The Chilean oyster becomes sexually mature at the beginning of the second year of life with a shell length of about 27 mm (7). After three years of growth under uniform conditions, 800 randomly chosen oysters were mass spawned in the laboratory. The temperature and salinity used in the experiment were within the range of those in the natural environment (10–18°C and 27–32 ppt). Although the use of mass spawning prevents one from knowing how many individuals contribute genes to the offspring obtained, the female contribution can be estimated by keeping track of the number in each brood of eyed larvae. Fecundity in *O. chilensis* ranges between 10,000 and 115,000, with an average of 60,000 (7). The number of larvae released, more than 8.2×10^6, indicates that at least 130 females contributed larvae. This number of females may be an underestimation because some of the eyed larvae released set within 5 min (7); thus this cohort cannot be treated as a product of restricted matings.

From an initial population size of 4050 randomly tagged juveniles grown at the Hueihue location (41°58′S,

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Table I

Heterozygote frequency and D values for four loci at three stages of the life cycle of Ostrea chilensis *(6, 18, and 30 months of age)*

Age (months)	Locus	O.H.	E.H.	*D*	(*P*)
6	LAP	0.419	0.398	0.053	NS
	GPI	0.581	0.458	0.283	*
	CA	0.645	0.617	0.046	NS
	PGM	0.155	0.167	−0.072	NS
18	LAP	0.338	0.457	−0.260	*
	GPI	0.373	0.389	−0.041	NS
	CA	0.514	0.607	−0.153	*
	PGM	0.247	0.383	−0.355	*
30	LAP	0.447	0.591	−0.243	*
	GPI	0.268	0.292	−0.082	NS
	CA	0.408	0.631	−0.353	*
	PGM	0.231	0.374	−0.382	*

Genotype frequencies were investigated using random samples of 150 oysters taken from each class interval. Each locus was tested individually, using the χ^2 goodness of fit test with *D* as an index of heterozygote deviation. Starch gel electrophoresis was used (18, 19) to score the loci leucine aminopeptidase (LAP, EC 3.4.1.1), glucose phosphate isomerase (GPI, EC 5.3.1.9), carbonic anhydrase (AC, EC 4.2.1.1), and phosphoglucomutase (PGM, EC 2.5.7.1).

O.H. = proportion of observed heterozygotes; E.H. = proportion of expected heterozygotes; *D* = heterozygote deviation index defined as (O.H. − E.H.)/E.H.; (*P*) = probability of the χ^2 goodness of fit to the Hardy Weinberg model (NS = nonsignificant; * = significant at alpha = 0.05).

73°30′W), the percentages of mortality at ages from 6 to 18 and 18 to 30 months were 25% and 17% respectively. At each class interval, 150 oysters were sampled without replacement. Neither significant deficiencies nor an excess of heterozygotes was found in three of four loci in the 6-month-old oysters; the exception was glucose phosphate isomerase (GPI), which showed an excess of heterozygotes (Table I). At 18 months, significant deficiencies of heterozygotes were found at LAP ($D = -0.260$), CA ($D = -0.150$), and PGM ($D = -0.355$) (Table I). In adult oysters (30 months), negative values of *D* were present at three of four analyzed loci, presenting significant values at LAP ($D = -0.243$), AC ($D = -0.353$), and PGM ($D = -0.382$) (Table I). The data showed that between the age of 6 and 18 months, three out of four loci studied showed a genotype-dependent mortality. This differential mortality produces a significant overall deficiency of heterozygosity in the cohort. One of the loci studied (GPI) showed an excess of heterozygotes at 6 months and neither an excess nor a deficiency of heterozygotes at 18 and 30 months; this result agrees with other studies carried out on this locus in natural populations of bivalve molluscs (1, 17, 18). High fecundity, external fertilization, and extensive larval dispersal—characteristics common to most of the bivalves molluscs—make it unlikely that inbreeding or the Wahlund effect could be the main cause of heterozygote deficiencies. The reproductive features of *O. chilensis* favor mechanisms such as inbreeding or Wahlund effect to act and produce heterozygote deficiencies. However, in accord with data for other marine bivalve species, we suggest that selection against heterozygotes is the most probable cause of the heterozygote deficiencies (2, 4, 11), because the deficiency is not evident at the juvenile stage but increases over time. We discarded inbreeding (which would affect the whole genome), Wahlund effect, aneuploidy, and null alleles as possible causes for the heterozygosity deficiency in this cohort because a deficiency produced by these factors should be evident from the juvenile stage and not necessarily increase over time (2, 5, 8, 9, 10).

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